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G. A. Marvin

University of North Alabama

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ARTICLE

Acute physiological response by the plethodontid salamander *Eurycea cirrigera* (Southern Two-lined Salamander) to predation stress from alarm chemicals and predator kairomones

G.A. Marvin

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Abstract: Plethodontid salamanders may reduce predation risk via behavioral responses to predator kairomones and alarm chemicals from injured salamanders. However, it not known whether such predator cues prompt acute physiological responses, which may enhance arousal and the physical ability to escape from a predator. I examined whether predator chemical cues elicit an acute cardiac response in *Eurycea cirrigera* (Green, 1831) (Southern Two-lined Salamander). I compared heart rates before and after exposure to the odor of the large predatory *Pseudotriton ruber* (Sonnini de Manoncourt and Latreille, 1801) (Red Salamander) and exposure to alarm chemicals from homogenized skin of conspecifics. For two controls, I compared heart rates before and after exposure to the odor of live conspecifics and the odor of the large non-predatory *Plethodon mississippi* Highton in Highton, Maha and Maxson, 1989 (Mississippi Slimy Salamander). Compared with resting values, heart rates significantly increased in response to predator kairomones (mean rate increased 10.9% after 2 min and 12.7% after 5 min) and alarm chemicals from conspecifics (mean rate increased 12.0% after 2 min and 14.5% after 5 min). In contrast, heart rates after exposure to each control odor did not significantly differ from resting values. Results demonstrate an acute cardiac response to chemical cues indicative of either a predator or a predation event.

Key words: alarm chemical, cardiac, *Eurycea cirrigera*, kairomone, predation, Southern Two-lined Salamander, stress response.

Résumé : Les salamandres de la famille des pléthodontidés pourraient réduire leur risque de prédation par des réactions comportementales aux kairomones de prédateurs et à des signaux d'alarme chimiques provenant de salamandres blessées. Il n'est toutefois pas établi que de tels signaux de prédateurs déclenchent des réactions physiologiques aiguës susceptibles de rehausser l'éveil et la capacité physique d'échapper à un prédateur. J'ai vérifié si des signaux chimiques de prédateurs provoquent une réaction cardiaque aiguë chez d'*Eurycea cirrigera* (Green, 1831) (salamandre à deux lignes du Sud). J'ai comparé leurs rythmes cardiaques avant et après l'exposition à l'odeur de *Pseudotriton ruber* (Sonnini de Manoncourt et Latreille, 1801) (salamandre rousse), une espèce de grandes salamandres prédatrices, et l'exposition à des signaux d'alarme chimiques d'échantillons de peau homogénéisée de conspécifiques. Pour deux témoins, j'ai comparé les rythmes cardiaques avant et après l'exposition à l'odeur de conspécifiques vivants et à l'odeur de *Plethodon mississippi* Highton in Highton, Maha and Maxson, 1989 (salamandre gluante du Mississippi), une espèce de grandes salamandres non prédatrices. Comparativement aux valeurs au repos, les rythmes cardiaques augmentent significativement en réaction aux kairomones de prédateurs (augmentation moyenne du rythme cardiaque de 10,9 % après 2 min et de 12,7 % après 5 min) et aux signaux d'alarme chimiques de conspécifiques (augmentation moyenne de 12,0 % après 2 min et de 14,5 % après 5 min). En comparaison, les rythmes cardiaques après l'exposition aux odeurs des deux témoins ne sont pas significativement différents des valeurs au repos. Les résultats démontrent la présence d'une réaction cardiaque aiguë à des signaux chimiques indiquant soit la présence d'un prédateur ou un évènement de prédation. [Traduit par la Rédaction]

Mots-clés : signal d'alarme chimique, cardiaque, *Eurycea cirrigera*, kairomone, prédation, salamandre à deux lignes du Sud, réaction de stress.

Introduction

Animals have diverse tactics to decrease the likelihood of predation. Individuals may use predator-avoidance mechanisms to avoid the foraging areas of predators and anti-predator mechanisms to reduce the probability of predation when they are within the perceptual fields of predators (Brodie et al. 1991). Predator-avoidance mechanisms include behavioral responses to predators such as hiding in refuges, altering foraging, and changing activity level or period (e.g., Smith 1979; Watson et al. 2004; Epp and Gabor 2008; Gildemeister et al. 2017). Anti-predator mechanisms include morphological traits (e.g., spines and shells), behavioral responses to predators (e.g., alterations in posture, immobility, biting, and fleeing), and physiological responses to predators (e.g., autotomy of an appendage and secretion of distasteful chemicals) that increase the probability of surviving an attack (e.g., Brodie 1977; Ducey and Brodie 1983; Dowdey and Brodie 1989). Predator-avoidance and anti-predator mechanisms that involve quick alterations in physiology and behavior are likely acute stress responses to predator cues (e.g., Sunardi and Manatunge 2007; Steiner and Van Buskirk 2009; Davis and Gabor 2015).

Stress-induced changes in physiology and behavior are often similar among vertebrate animals and result from the vertebrate stress response (Lima 1998; Denver 2009). Both the sympathetic

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G.A. Marvin. Department of Biology, University of North Alabama, Box 5048, 1 Harrison Plaza, Florence, AL 35632-0002, USA.
Email for correspondence: gamarvin@una.edu.

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nervous system – epinephrine system and the hypothalamic–pituitary–adrenal (hypothalamic–pituitary–inter-renal in amphibians) system facilitate neuroendocrine responses to environmental stressors (Sapolsky 2002; Chrousos 2009). Activation of the sympathetic nervous system – epinephrine system during stress produces many acute physiological adjustments by an individual (i.e., the fight-or-flight response), such as an increase in blood glucose and the rate of oxygen transport to tissues (e.g., nerve and muscle), which may enhance physical performance (Jansen et al. 1995; Sapolsky 2002). Activation of the hypothalamic–pituitary–adrenal (hypothalamic–pituitary–inter-renal) system by stress often increases blood plasma levels of glucocorticoids (GCs; cortisol or corticosterone) that can potentiate the effects of epinephrine, increase glucose availability, and enhance arousal and escape behavior (Sapolsky 2002; Chrousos 2009). Thus, the measurement of changes in plasma GC level is one method to quantify the physiological response by vertebrate species to potential stressors, such as predators (Dantzer et al. 2014; Woodley 2017).

In many fish and amphibian species, the detection of predator cues by an individual often elicits behavioral responses to avoid predators. Avoidance of areas with predator odors (kairomones) occurs in many species (e.g., Petranka et al. 1987; Roudebush and Taylor 1987; Kats and Sih 1992; Cupp 1994; Flowers and Graves 1997; Ferrari et al. 2010). Similarly, individuals that detect alarm chemicals released from injured individuals may increase refuge use or avoid areas with such chemicals (e.g., Lutterschmidt et al. 1994; Marvin and Hutchison 1995; Chivers and Smith 1998). Individuals that detect predator cues may also decrease activity (e.g., Smith 1979; Mathis et al. 2003; Epp and Gabor 2008) and alter foraging and mating behavior to reduce exposure to predators (Whitham and Mathis 2000; Sullivan et al. 2002; Watson et al. 2004; Johnson and Sullivan 2014; Fonner and Woodley 2015; Gildemeister et al. 2017). In addition to adaptive behavioral responses to predation stress, acute physiological responses may enhance some anti-predator mechanisms. For example, individuals of some fish species increase gill ventilation rate and (or) metabolic rate in response to predator chemical cues, which may enhance oxygen transport, aerobic performance, and the ability to escape from a predator (e.g., Gibson and Mathis 2006; Sunardi and Manatunge 2007; Sanches et al. 2015). The predation stress hypothesis predicts that the release of GCs coordinates behavioral and physiological responses by prey to avoid predation (Lima 1998). However, although plasma GC levels increase during stress in some vertebrates (Rehnberg and Schreck 1987; Toa et al. 2004; Ricciardella et al. 2010; Woodley and Lacy 2010; Narayan and Hero 2014), a lack of changes in GC levels in response to some predator cues does not support the predation stress hypothesis in some amphibians (Davis and Gabor 2015; Fonner and Woodley 2015).

In some amphibian species, individuals decrease locomotor activity but do not exhibit a concomitant increase in GC levels when exposed to some predator chemical cues. Tadpoles of the frog species *Rana sylvatica* LeConte, 1825 (Wood Frog) and *Rana clamitans* Latreille in Sonnini de Manoncourt and Latreille, 1801 (Green Frog) reduce swimming activity, but show a decrease in whole body corticosterone content, in response to an alarm pheromone released from conspecifics (Fraker et al. 2009). In the aquatic plethodontid salamander *Eurycea nana* Bishop, 1941 (San Marcos Salamander), individuals reduce activity in response to kairomones from two predatory fish species (Epp and Gabor 2008; Davis and Gabor 2015) but only exhibit a corresponding increase in plasma corticosterone to one of these species (Davis and Gabor 2015). Similarly, individuals of the semiaquatic plethodontid salamander *Desmognathus ochrophaeus* Cope, 1859 (Allegheny Mountain Dusky Salamander) reduce locomotor activity in response to kairomones from a predatory salamander species but show no concomitant change in plasma corticosterone (Fonner and Woodley 2015). Thus, because predator-avoidance and anti-predator mechanisms may not correspond to changes in GC levels for some amphibians, researchers must measure alternative physiological variables to quantify physiological responses by these species to predator cues.

In this study, I examined heart rate to assess whether individuals of the semiaquatic plethodontid salamander *Eurycea cirrigera* (Green, 1831) (Southern Two-lined Salamander) exhibit an acute physiological response to predation stress. I performed a controlled laboratory experiment to test the hypothesis that salamander heart rate increases after exposure to chemical cues indicative of either a predator or a predation event. Elevated heart rate might increase the rate of oxygen transport to tissues and improve aerobic capacity, which may enhance some anti-predator mechanisms (e.g., fleeing ability). As two experimental stimuli, I used alarm chemicals from homogenized skin of conspecifics and odor from the predatory *Pseudotriton ruber* (Sonnini de Manoncourt and Latreille, 1801) (Red Salamander). As two control stimuli, I used odor from live conspecifics and odor from the non-predatory *Plethodon mississippi* Highton in Highton, Maha and Maxson, 1989 (Mississippi Slimy Salamander). In addition, experimental results further test hypotheses that predict individuals of the genus *Eurycea* Rafinesque, 1822 (Brook Salamander) respond to alarm chemicals (Marvin et al. 2004) and predator kairomones (Whitham and Mathis 2000; Hickman et al. 2004; Epp and Gabor 2008; Davis and Gabor 2015; Davis et al. 2017; Marvin and Cupp 2018), and discriminate between the odors of predatory versus non-predatory species (Whitham and Mathis 2000; Hickman et al. 2004; Epp and Gabor 2008; Marvin and Cupp 2018).

Materials and methods

Rationale for choice of species

Because individuals of some *Eurycea* species (e.g., *Eurycea wilderae* Dunn, 1920 (Blue Ridge Two-lined Salamander); closely related to *E. cirrigera*) avoid substrates with alarm chemicals from homogenized skin of conspecifics (Marvin et al. 2004), individuals of *E. cirrigera* may exhibit a stress response to such chemical cues. Individuals of the large plethodontid salamander *P. ruber* are predators of smaller salamanders including *E. cirrigera* (Dunn 1926; Bishop 1941; Gustafson 1993). Individuals of *E. cirrigera* avoid substrates with odor from individuals of *P. ruber* (Marvin and Cupp 2018), which indicates they respond behaviorally to kairomones from this species. Although individuals of the large *Plethodon* Tschudi, 1838 (Woodland Salamander) species may occasionally consume very small salamanders (Oliver 1967; Powders 1973; Powders and Tietjen 1974), they primarily eat invertebrates (e.g., Hamilton 1932; Pope 1950; Davidson 1956; Oliver 1967; Mitchell and Taylor 1986; Milanovich et al. 2008; Hutton et al. 2017). Because individuals of *E. cirrigera* do not avoid substrates with the odor of the large *Plethodon* (Marvin and Cupp 2018), I used the odor of sympatric *P. mississippi* as a control stimulus in the present study. Whereas metamorphosed individuals of *E. cirrigera* and *P. ruber* are semiaquatic and often live in streamside or seep habitats, individuals of *P. mississippi* are strictly terrestrial and primarily inhabit woodlands (Highton 1995). However, metamorphosed individuals of *E. cirrigera* and *P. ruber* often traverse terrestrial habitat in woodlands around streams and seeps on moist or rainy evenings. Thus, metamorphosed individuals of *E. cirrigera* may encounter odors from both of these larger salamander species when they move away from streams and seeps.

Animal collection and care

In March of 2018, I collected 31 adult *E. cirrigera* (16 males, 15 females), 4 adult *P. ruber* (2 males, 2 females), and 4 adult *P. mississippi* (2 males, 2 females) from the same locality (34°62.15′N, 87°79.75′W; USNG 16SDD26863133) in northwestern Alabama, USA. Sex was determined by presence of a mental gland in males of *P. mississippi* and presence of eggs in females of *E. cirrigera* and *P. ruber*. Presence of *P. mississippi* (i.e., not *Plethodon glutinosus* (Green, 1818) (Northern Slimy Salamander), a closely re-

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lated cryptic species) from this locality was verified by R. Highton (University of Maryland, College Park, Maryland, USA; personal communication, 2010) based on an electrophoretic study of blood proteins. Mean ± 1 SD (range) snout to posterior angle of vent length was 39.1 ± 3.1 mm (34–45 mm) for *E. cirrigera*, 85.7 ± 6.2 mm (79–92 mm) for *P. ruber*, and 71.2 ± 2.4 mm (69–76) mm for *P. mississippi*. Mean ± 1 SD (range) body mass was 0.95 ± 0.16 g (0.8–1.4 g) for *E. cirrigera*, 10.70 ± 2.11 g (8.4–13.4 g) for *P. ruber*, and 6.15 ± 0.39 g (5.8–6.8 g) for *P. mississippi*. Animals were cared for in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Academy Press, 2101 Constitution Avenue NW, Washington, DC 20055, USA). Individuals were kept in separate, clean, disinfected housing containers (ca. 14 cm × 14 cm × 3.5 cm) within an environmental chamber at 20.6 °C (to match room temperature during experimental trials) with a 12 h light : 12 h dark photoperiod for 4 weeks prior to experimental trials. I replaced moist paper towel substrates in housing containers and fed salamanders to satiation once each week. Individuals of *E. cirrigera* were fed vestigial wing *Drosophila* Fallén, 1823, whereas individuals of *P. ruber* and *P. mississippi* were fed small redworms (*Eisenia fetida* (Savigny, 1826)). With a salamander diet of invertebrates, I minimized the potential effect of variation in diet (i.e., with salamanders versus invertebrates as prey) on salamander odors (e.g., Madison et al. 1999; Sullivan et al. 2004). Under approval of the University of North Alabama Institutional Animal Care and Use Committee, I released all live salamanders in good health at the capture locality at the conclusion of the study. To reduce the potential risk of introducing pathogens into wild populations from individuals held in captivity, I disinfected all equipment and salamander containers with 70% ethanol for at least 1 min before and after use.

Experimental protocol

I conducted this research under approval of the University of North Alabama Institutional Animal Care and Use Committee, but the Committee does not issue protocol approval numbers. I conducted four experimental trials. During each trial, I determined heart rate for 23 experimental individuals of *E. cirrigera* (12 males, 11 females) before and after exposure to one of four test odors (i.e., two control and two experimental). Control odors were from live conspecific individuals or live individuals of *P. mississippi*. Experimental odors were from the homogenized skin of recently killed conspecifics or from live individuals of *P. ruber*. For each individual *E. cirrigera*, I used a unique sequence (i.e., 1 of 24 possible) for presentation of the four test odors across the four experimental trials. For each individual, the time between exposures to different odors in different trials was 7 days. I conducted each experimental trial at 20.6 °C from the 2nd to the 6th hour of the scotophase.

To obtain the odor of live salamanders for an experimental trial, I produced aqueous body rinses from two individuals (one male, one female) of each species. To produce the body rinse for a species, I placed randomly selected individuals in separate clean glass dishes (9.0 cm diameter × 1.5 cm for *E. cirrigera*, 20 cm diameter × 7 cm for each large species). Each dish had a volume of distilled water that was proportional to the individual's mass (4 mL per 1 g salamander mass). Each salamander remained in its closed dish for 72 h at 20.6 °C. I filtered the rinse water from each dish through a 1 μm filter to remove any large solid material. To increase the volume of the filtered salamander rinse water, I mixed it with an equal volume of distilled water. To reduce possible individual odor effects, I then mixed equal volumes of the rinse water obtained from the two conspecific individuals. Within 280 min, I used the rinse water from each species in an experimental trial.

Prior to the experiment, I randomly selected eight individuals of *E. cirrigera* (four males, four females) as the source of odor from live conspecific individuals and alarm chemicals from homogenized conspecific skin. For the odor of homogenized conspecific skin in an experimental trial, I killed two randomly selected individuals (one male, one female) of *E. cirrigera* by decapitation. I removed the skin from each salamander carcass and homogenized the skin tissue in distilled water (60 mL per 1 g tissue). I filtered the homogenized-tissue solution through a 1 μm filter to remove large solid material. To reduce possible individual odor effects, I then mixed equal volumes of the filtered homogenized-tissue solution obtained from the two conspecific individuals. Within 280 min, I used water with odor from homogenized conspecific skin in an experimental trial.

At 24 h prior to each experimental trial, I transferred the 23 experimental individuals of *E. cirrigera* from their housing containers to separate clean, disposable, plastic petri dishes (50 mm × 12 mm) with 3 mL of distilled water in each dish. To limit the salamander's vertical movement to about 5 mm, I placed a 48 mm diameter piece of clean, stiff plastic screen (3 mm × 3 mm mesh, 1 mm thick) above the salamander inside the dish. To elevate and support the plastic screen, I previously attached three 5 mm plastic beads with silicon sealant at equidistant intervals near the edge. I placed a clean, 12.5 g, 35 mm diameter washer on top of the screen to maintain screen position in the dish. I then placed a clean lid on each individual's dish to reduce water loss and evaporative cooling. To allow introduction of a test odor solution into each dish during an experimental trial (as described below), I drilled a 3 mm hole in the center of each dish lid prior to the experiment.

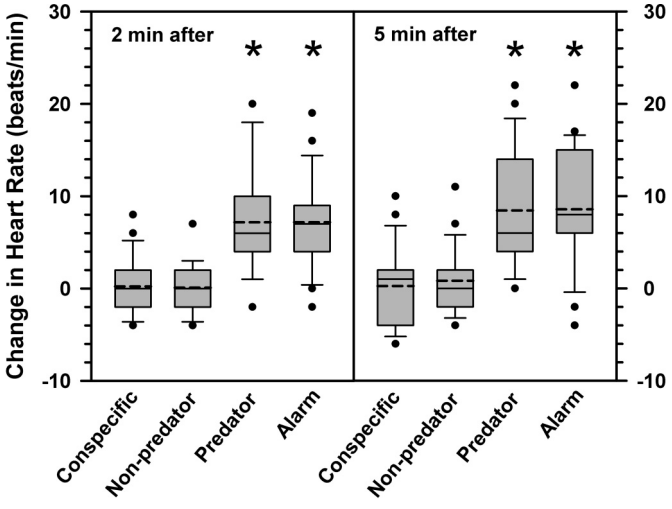
For observation of an individual's heart rate in an experimental trial, I gently placed the salamander's dish on top of a glass plate mounted above a video camera (Panasonic WV-CD 110A) with a 16 mm COSMICAR TV lens. The video camera provided a magnified (27×) ventral view of the salamander on a video monitor (32 cm × 42 cm). Ambient light in the experimental room was 1 lx. To illuminate the salamander sufficiently to view the heart through the ventral skin, I mounted a small LED light 2 m from the side of the petri dish at a height of 0.52 m. A light meter showed that the LED light provided 300 lx illumination of the salamander's skin surface. With the light directed toward the lateral surface of the salamander (at a 15° angle above the ventral surface), the ventral skin was translucent enough that the beating heart was unmistakably visible on the video monitor. Continuous readings via a small thermocouple probe in the water of the dish verified that any heat emitted from the light and video camera did not alter the water temperature during a trial. I waited 3 min before making observations of salamander heart rate.

From behind a blind, I observed the salamander's heartbeat on the video monitor. I recorded the number of heartbeats (estimated to the nearest half cycle) during a 30 s interval to calculate the resting heart rate prior to odor exposure (i.e., number of beats/min = twice the number of beats in 30 s). Immediately after observation of the resting heart rate, I used a pipette to introduce 2 mL of a test odor solution (i.e., salamander rinse water or alarm chemicals from homogenized skin) into the petri dish through the 3 mm hole in the center of the lid. At both 2 and 5 min intervals after introduction of the test odor solution, I again recorded the number of heartbeats during a 30 s interval to determine the individual's heart rate.

Statistical analyses

For each of the four test odors, I calculated the change in heart rate (i.e., the resting rate immediately prior to odor exposure was subtracted from the heart rate after exposure) for each individual of *E. cirrigera* at both 2 and 5 min after exposure to the odor. Data on changes in heart rate met assumptions of parametric statistical tests. To test for the effects of odor type and time (after odor exposure) on the change in heart rate, I performed two-way repeated-measures analysis of variance (ANOVA) to analyze heart-rate changes for individuals at 2 and 5 min after exposure to each

Fig. 1. Change in heart rate for 23 adult *Eurycea cirrigera* (Southern Two-lined Salamander) at 2 and 5 min after exposure to four test odors at 20.6 °C. Conspecific refers to the odor of live adult conspecifics; Non-predator refers to the odor of live adult *Plethodon mississippi* (Mississippi Slimy Salamander), which is a non-predatory species; Predator refers to the odor of live adult *Pseudotriton ruber* (Red Salamander), which is a predatory species; Alarm refers to alarm chemicals from homogenized skin of conspecifics. Change in heart rate was significantly greater in response to both Predator and Alarm than to either Conspecific or Non-predator (two-way repeated-measures ANOVA; $F_{[3,22]} = 26.19$, *$P < 0.001$) and was significantly greater at 5 min than at 2 min (two-way repeated-measures ANOVA; $F_{[1,22]} = 7.66$, $P = 0.011$). Box plots show minimum, maximum, median (solid horizontal line), mean (broken horizontal line), and percentiles (10th, 25th, 75th, and 90th).



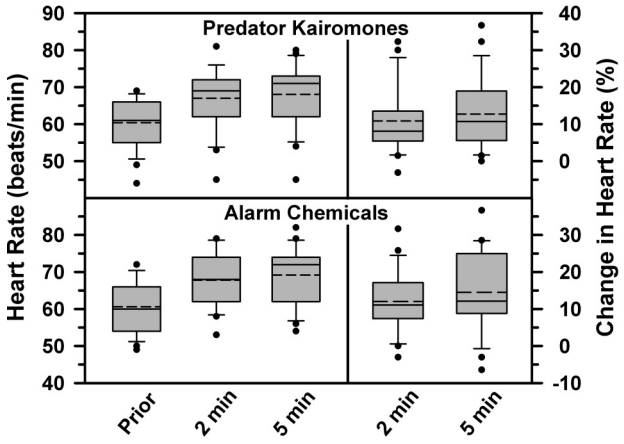
of the four odors. The Holm–Šidák method was used for multiple comparison procedures on both odor and time factors. To examine whether heart rates at 2 and 5 min after exposure to each control odor differed from resting rates prior to exposure, I performed one-way repeated-measures ANOVA on $\log_{10}$-transformed values because the raw data did not meet assumptions of parametric tests. For statistical tests, I used SigmaPlot® version 11 software with a significance level of $P \leq 0.05$.

Results

The change in heart rate for individuals was significantly different in response to the four test odors (two-way repeated-measures ANOVA, $F_{[3,22]} = 26.19$, $P < 0.001$; Fig. 1). The change in heart rate was significantly greater after exposure to the odor of either live individuals of *P. ruber* ($t = 6.33$, $P < 0.001$) or homogenized skin of conspecific individuals ($t = 6.38$, $P < 0.001$) than to the odor of live conspecific individuals (Holm–Šidák multiple comparisons; Fig. 1). Similarly, the change in heart rate was significantly greater after exposure to the odor of either live individuals of *P. ruber* ($t = 6.15$, $P < 0.001$) or homogenized skin of conspecific individuals ($t = 6.20$, $P < 0.001$) than to the odor of live individuals of *P. mississippi* (Holm–Šidák multiple comparisons; Fig. 1). Heart-rate change after exposure to the odor of live individuals of *P. ruber* did not significantly differ from heart-rate change after exposure to odor of homogenized conspecific skin (Holm–Šidák multiple comparisons, $t = 0.05$, $P = 0.957$; Fig. 1). Heart-rate change after exposure to the odor of live individuals of *P. mississippi* did not significantly differ from heart-rate change after exposure to the odor of live conspecific individuals (Holm–Šidák multiple comparisons, $t = 0.18$, $P = 0.979$; Fig. 1).

The change in heart rate significantly varied with time after exposure to odors (two-way repeated-measures ANOVA, $F_{[1,22]} =$ 7.66, $P = 0.011$) and was significantly greater at 5 min than at 2 min (Holm–Šidák method, $t = 2.77$, $P = 0.011$; Figs. 1 and 2). There was no significant interaction between the factors of odor and time (two-way repeated-measures ANOVA, $F_{[3,22]} = 1.52$, $P = 0.218$). Log_{10}-transformed heart rates at 2 and 5 min after exposure to the odor of either live conspecific individuals or live individuals of *P. mississippi* did not significantly differ from $\log_{10}$-transformed resting rates (one-way repeated-measures ANOVA: $F_{[2,22]} = 0.012$, $P = 0.988$ and $F_{[2,22]} = 1.16$, $P = 0.323$, respectively).

Fig. 2. Heart rate for 23 adult *Eurycea cirrigera* (Southern Two-lined Salamander) at 20.6 °C prior to and after exposure (at 2 and 5 min) to predator kairomones from live adult *Pseudotriton ruber* (Red Salamander) and to alarm chemicals from homogenized skin of conspecifics. Percent change in heart rate for individuals at 2 and 5 min after exposure is presented on the right side of figure. Box plots show minimum, maximum, median (solid horizontal line), mean (broken horizontal line), and percentiles (10th, 25th, 75th, and 90th).



Discussion

Results support the hypothesis that heart rate for individual *E. cirrigera* increases after exposure to chemical cues indicative of either a predator or a predation event. Individuals displayed a significant increase in heart rate after exposure to either kairomones from a predatory salamander species or alarm chemicals from conspecifics (Fig. 1). The mean heart rate for individuals increased 10.9% after 2 min and 12.7% after 5 min when exposed to the odor of the predatory salamander *P. ruber* (Fig. 2). Similarly, mean heart rate for individuals increased 12.0% after 2 min and 14.5% after 5 min when exposed to the odor of homogenized conspecific skin (Fig. 2). The magnitude of this increase was less than half of the maximal ability, because the mean increase in heart rate for these individuals was 33.3% after forced maximal swimming performance over 5 m at 20.6 °C (unpublished data collected after completion of this study). Salamander heart rates after exposure to each control odor (i.e., from either live conspecific individuals or live individuals of *P. mississippi*) did not significantly differ from resting values, which indicates the elevated rates in response to each experimental odor were not a common response to chemicals from other salamanders. To my knowledge, this is the first study of a plethodontid salamander species to demonstrate (*i*) an acute cardiac response by individuals to predation stress and (*ii*) an acute physiological response by individuals to alarm chemicals from conspecifics. An acute cardiac response to either predator kairomones or alarm chemicals might elevate oxygen transport rate and the aerobic performance of tissues, which may improve the salamander's ability to elude a predator (e.g., perhaps via quicker reflex responses and better fleeing capability).

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Intra-guild predation often plays an important role in population dynamics and community structure (e.g., Hairston 1987; Polis et al. 1989). For plethodontid salamanders, responses to odors from intra-guild predators likely influence the structure of some streamside communities (Hairston 1986; Roudebush and Taylor 1987). Although the impacts of predation by larval *Pseudotriton* Tschudi, 1838 (Red Salamander genus) on growth and survivorship of larval *Eurycea* have been examined (Gustafson 1993), the importance of predation after metamorphosis is largely unknown. Because metamorphosed individuals of *E. cirrigera* avoid areas with kairomones from metamorphosed individuals of *P. ruber* (Marvin and Cupp 2018), such chemical cues elicit a behavioral response that may affect microhabitat choice. In addition to behavioral stress responses, results of the present study indicate physiological stress responses to kairomones from intra-guild predators may influence predator–prey interactions in plethodontid salamander communities. Because metamorphosed individuals of *E. cirrigera* are semiaquatic and often move through both aquatic and terrestrial habitats in woodlands, it may be beneficial for them to distinguish among odors from various salamander species and preferentially respond to odors from probable predators. My results indicate that individuals of *E. cirrigera* do not exhibit an acute cardiac response to the odor of large *Plethodon* individuals, which primarily eat small invertebrates (e.g., Hamilton 1932; Pope 1950; Davidson 1956; Oliver 1967; Mitchell and Taylor 1986; Milanovich et al. 2008; Hutton et al. 2017). These findings support the hypothesis that individual *E. cirrigera* distinguish between chemical cues from larger salamander species that are predatory versus non-predatory (Marvin and Cupp 2018). Similarly, individuals of several other plethodontid salamander species (*Desmognathus wrighti* King, 1936 (Pygmy Salamander), *Eurycea multiplicata* (Cope, 1869) (Many-ribbed Salamander), *E. nana*, *Plethodon angusticlavius* Grobman, 1944 (Ozark Zigzag Salamander)) differentially respond to odors from animal species that are likely predators of salamanders (Whitham and Mathis 2000; Hickman et al. 2004; Epp and Gabor 2008; Crane et al. 2012; Forester et al. 2019). Additionally, results of the present study indicate that individuals of *Eurycea* exhibit an acute physiological response to alarm chemicals which, in conjunction with behavioral responses (Marvin et al. 2004), might reduce an individual's predation risk by enhancing both predator-avoidance and anti-predator mechanisms.

As proposed for individuals of many fish species, an acute physiological response by individuals of *E. cirrigera* may enhance arousal and improve physical performance during encounters with predators by enhancing the rate of oxygen transport to tissues. Increased oxygen consumption in some fish species corresponds to increased opercular activity (Dalle Valle et al. 2003), and an increase in gill ventilation rate and (or) metabolic rate in response to predator cues occurs in various species. Opercular activity in fry of *Salmo salar* Linnaeus, 1758 (Atlantic salmon) increased significantly after exposure to predatory fish species (Hawkins et al. 2004, 2007). Opercular beat rates in *Etheostoma caeruleum* Storer, 1845 (rainbow darter) increased 15%–35% after exposure to alarm chemicals from conspecific and heterospecific fishes (Gibson and Mathis 2006). Gill ventilation rate in *Pseudorasbora parva* (Temminck and Schlegel, 1846) (topmouth gudgeon) increased 10.5% and 33.8% after exposure to predator odors and alarm chemicals, respectively, which may have supported a significant elevation of metabolic rate after exposure (Sunardi and Manatunge 2007). Alarm chemicals from conspecifics elicited a significant increase in both gill ventilation rate and plasma cortisol level in *Oreochromis niloticus* (Linnaeus, 1758) (Nile tilapia) (Barreto et al. 2010; Sanches et al. 2015). Metabolic rates of larval *Pimephales promelas* Rafinesque, 1820 (fathead minnow) were significantly elevated in the presence of alarm chemicals (Robison et al. 2018).

Compared with fishes, there are relatively few studies of physiological responses by amphibians to predation stress. Oxygen consumption by tadpoles of *Rana temporaria* Linnaeus, 1758 (European Frog) increased 16.8% after short-term exposure to kairomones from predatory dragonfly larvae (Steiner and Van Buskirk 2009). In contrast, tadpoles of *Bufo arabicus* Heyden, 1827 (= *Sclerophrys arabica* (Heyden, 1827); Arabian Toad) significantly reduced respiration rate in response to kairomones from dragonfly larvae (Barry and Syal 2013). Although numerous studies on plethodontid salamander species demonstrated behavioral responses by individuals to predator chemical cues (e.g., Roudebush and Taylor 1987; Cupp 1994; Marvin et al. 2004; Watson et al. 2004; Forester et al. 2019), only two studies documented an acute physiological response (Crane et al. 2012; Davis and Gabor 2015). Individuals of the plethodontid salamander *P. angusticlavius* increased activity and metabolic rate in response to kairomones from a predatory mammal species (Crane et al. 2012). In the plethodontid salamander *E. nana*, individuals exhibited an increase in plasma corticosterone after exposure to kairomones from a predatory fish species with a low encounter frequency (Davis and Gabor 2015). Chronic exposure to kairomones from a predatory snake species reduced the rate of tail regeneration in the plethodontid salamander *D. ochrophaeus* (Payette and Sullivan 2019). Exogenous application of corticosterone produced a 12% increase in oxygen consumption rate for individuals of *Plethodon shermani* Stejneger, 1906 (Redlegged Salamander) (Wack et al. 2012). Although some acute environmental stressors increased plasma corticosterone in *D. ochrophaeus* (Ricciardella et al. 2010), kairomones from a predatory salamander species did not elevate plasma corticosterone level (Fonner and Woodley 2015). Because GC levels do not significantly increase during predation stress in some amphibians, such as some plethodontid salamanders (Woodley 2017), the assessment of physiological responses by these species to predator cues requires the measurement of alternative physiological variables.

Unlike individuals of other vertebrate taxa, metamorphosed individuals of plethodontid salamander species lack a specialized respiratory surface for gas exchange (i.e., gills or lungs) and must obtain oxygen across the skin and buccopharyngeal mucosa (Dunn 1926; Whitford and Hutchison 1965). Because these salamanders can only physiologically alter ventilation of the buccopharyngeal mucosa to increase oxygen uptake and blood oxygenation (Whitford and Hutchison 1965), individuals must primarily increase heart rate to enhance cutaneous blood flow and the rate of oxygen transport to tissues (Whitford 1968). Thus, the measurement of heart rate may allow researchers to quantify acute physiological responses by plethodontid salamander species to potential stressors. My visual method to determine heart rate performed very well in a small plethodontid salamander species with relatively little pigmentation in the ventral skin, but it would not work for larger species or those with highly pigmented ventral skin. For some larger plethodontid salamanders, it is possible to see (with sufficient magnification and illumination) pulsatile blood flow through pedal arterioles (personal observations), which might be used to visually determine heart rate. Alternatively, the "Buddy device", which uses an infrared beam to detect distortions of the skin surface from heart movements, can be used to measure heart rate in some salamanders (Catenazzi 2016). In addition to heart rate, studies that measure acute changes in metabolic rate may quantify physiological responses by plethodontid salamanders to potential stressors such as predator cues (Crane et al. 2012).

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